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January 24, 2005

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APPLICATION NUMBER: *60/528,140*
FILING DATE: *December 09, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/41282*

Certified by

Jon W. Dudas

Under Secretary of Commerce
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PTO/SB/16 (08-03)
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19587 U.S. PTO
60/528140

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 325774971 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Donata R.	Sizemore	St. Louis, Missouri

Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Expression of Anthrax and Plague Antigens

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS ***(check all that apply)***

☑ Specification *Number of Pages* 1
☐ CD(s), Number
☐ Drawing(s) *Number of Sheets*
☐ Other (specify)
☐ Application Date Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.
☑ A check or money order is enclosed to cover the filing fees. (check no. 4722)
☐ The Director is herby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-0268
☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): 160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.
☑ Yes, the name of the U.S. Government agency and the Government contract number are: Department of Defense (JVAP), subcontract under DynPort Vaccine Co., LLC, no. DPSC-02-02257

[Page 1 of 2]

Respectfully submitted,

SIGNATURE Leon R. Yankwich

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TELEPHONE 617-374-3700

Date December 9, 2003

REGISTRATION NO. 30,237 *(if appropriate)*

Docket Number: AVA-440.0 PRV

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Additional Page

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Docket Number AVA-440.0 PRV

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Number 1 of 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Sizemore et al.	
Serial No.:	(not yet assigned)	Examiner:
Filed:	(concurrently herewith)	Art Unit:
Entitled:	Expression of Anthrax and Plague Antigens	

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Mail Stop Provisional Application
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF EXPRESS MAIL

The undersigned hereby certifies that this certificate and the papers and fees identified below as being transmitted herewith are being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated below and are addressed to: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

The following items are transmitted herewith:

1. Provisional Application For Patent Coversheet (2 pages) (in duplicate)
2. Specification (1 page)
3. Check no. 4922 to cover filing fee
4. return post card

Express Mail # EV 325774971 US

date of deposit: December 9, 2003

Stephanie L. Leicht

Construction and Screening of Anthrax and Plague Vaccine Candidates Expressed in an Attenuated ΔphoP/Q *Salmonella typhimurium* Strain

Donata R. Sizemore[1], Beth Warner[1], Julie Lawrence[1] and Kevin Killeen[2]. AVANT Immunotherapeutics, Inc St. Louis, MO 63114[1] and Needham, MA 02494[2]

Efficacy studies evaluating anthrax and plague vaccines in animals have shown that antibodies specific for PA (anthrax) and F1 and V antigen (plague) are potential correlates with protection. This parameter was used to select potential attenuated ΔphoP/Q *Salmonella typhimurium* constructs expressing PA, F1, V, F1-V or fragments of PA and V from Asd^+ balanced-lethal plasmids to maintain stable antigen producing Salmonella vectors in the absence of antibiotic selection. Various plasmid expression vectors were evaluated that either secreted the antigen, placed the antigen in the outer membrane or expressed the antigen in the bacterial cell cytoplasm. To evaluate immunogenicity, mice were orally inoculated with frozen inocula of 1×10^9 CFU on days 0 and 14. Retained inocula samples were evaluated by Western blot after feeding to demonstrate the desired antigen was still being expressed. Naïve mice and mice inoculated with a live bacterial vector expressing no antigen were included as controls. Serum was collected at day zero prior to immunization from 10 mice and again at 2 and 4 weeks post-boost and evaluated for IgG antibodies against Salmonella vector and target antigen. Two strains were found to induce high levels of serum IgG specific to the expressed heterologous antigen. The strains were M020, which expresses soluble F1-V in the bacterial cell cytoplasm and M023, which expresses soluble V at extremely high levels in the bacterial cell cytoplasm. End-point titers (reciprocal of highest dilution above 0.1 OD450 as measured by ELISA) for serum IgG specific to V antigen for M020 vaccinated mice ranged from 100-1600 at 2 weeks post-boost and 400- 6400 at 4 weeks post-boost. End-point titers for M023 ranged from 1600-6400 at 2 weeks post-boost and 800- 25600 at 4 weeks post-boost. End-point titers for serum IgG specific to F1-V for M020 ranged from 100-6400 at 2 weeks post-boost and 400 to 6400 at 4 weeks post-boost. Currently six additional candidates are undergoing testing. Based on these early comparisons the most promising results were obtained from cytoplasmic localized F1-V and V antigens.

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